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二倍体铁破锣的核型及四倍体细胞型的首次发现*

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Correction of karyotype of diploid *Beesia calthifolia* and discovery of a tetraploid cytotype

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Abstract In this paper, the chromosomes of *Beesia calthifolia* were re-examined. In the 40 plant individuals of the population from Xinning County, Hunan Province, central China, one was found to be tetraploid with the karyotype formula of $2n = 4x = 32 = 16m + 8sm + 4st + 4t$, and the remaining were all found to be diploids with the karyotype formula of $2n = 2x = 16 = 8m + 4sm + 2st + 2t$. All the 10 individuals of the population from Cangshan Mountain, Dali City, Yunnan Province, southwestern China, were unexpectedly found to be tetraploids with the karyotype formula of $2n = 4x = 32 = 16m + 8sm + 4st + 4t$. Tetraploid cytotype was reported in this species for the first time. Based on the results and those previously reported, it is considered that there may exist some errors in the results of the karyotype analysis of this species previously reported by Shang(1985). He might have at least mistakenly recognized the centromere position of the fourth pair of chromosomes. This pair of chromosomes should have subterminal rather than median centromeres. Furthermore, the karyotypic differences of *B. calthifolia* and *B. deltophylla* were analyzed and the systematic position of the genus *Beesia* was discussed in detail.

Key words *Beesia calthifolia*; Karyotype; Systematic position

Beesia Balf. et W. W. Smith is a small genus of the Ranunculaceae, including only two very closely related species. *B. calthifolia* (Maxim. ex Oliv.) Ulbr. is widely distributed in China and northern Myanmar, while *B. deltophylla* C. Y. Wu ex Xiao is endemic to southeastern Xizang's Medog County of China(Xiao, 1979).

Both species have been cytologically studied and found to have the same chromosome number of $2n = 16$(Yang *et al.*, 1995 ; Shang, 1985), but their karyotypes reported by those authors are somewhat different. Shang(1985) reported the karyotype of *B. calthifolia* as consisting of 10 m-, 4 sm- and 2 t-chromosomes, with five large pairs(the first to fifth) having median centromeres(the fifth pair having an arm ratio of 1.22), two pairs(the sixth and the eighth) having submedian centromeres, and one pair(the seventh pair) having subterminal centromeres. Yang *et al.*(1995) reported the karyotype of *B. deltophylla* as consisting of 10 m-, 4 st- and 2 t-chromosomes, with five pairs(the first to fifth) having median centromeres(the fifth pair having an arm ratio of 1.66), two pairs(the sixth and seventh) having subterminal centromeres, and one pair (the eighth) having terminal centromeres. As pointed out in the paper on the karyotype of *B. deltophylla* cited above, there

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may exist some errors in Shang's results of the karyotype analysis of *B. calthifolia*. He might have at least mistakenly recognized the position of the centromeres of the fourth pairs of chromosomes. This pair of chromosomes may have subterminal rather than median centromeres, the satellites on the short arms indicated by Shang being actually short arms. Therefore, it is quite necessary to re-examine the chromosomes of this species to determine its karyotypic constitution. The paper is to report the results of the re-examination and the unexpected discovery of a tetraploid cytotype in this species.

1 Materials and Methods

Two populations, one from Zhiyunshan Mountain, Xinning County, Hunan Province, central China, which includes 40 plant individuals, the other from Cangshan Mountain, Dali City, Yunnan Province, southwestern China, which includes 10 plant individuals, were collected from the field and then transplanted in the greenhouse of the Laboratory of Systematic and Evolutionary Botany, Institute of Botany, the Chinese Academy of Sciences in Beijing and in that of the Botanic Garden, Kunming Institute of Botany, the Chinese Academy of Sciences in Kunming, respectively. The voucher specimens, Luo Yi-bo 945 and Yang Qin-er 9415, were both deposited in the Herbarium, Institute of Botany, the Chinese Academy of Sciences(PE).

For the observation of the chromosomes, actively growing roots were harvested and then pretreated with 0.1% colchcine for 2.5 hours at room temperature. They were then fixed in Carnoy's fluid(absolute ethanol: glacial acetic acid = 3:1) at 4℃ for 30 minutes. After being macerated in the mixture of 1 mol/L HCl and 45% acetic acid(1:1) at 60℃ for three minutes, they were then stained in 1% aceto-orcein and squashed.

Karyomorphological classification of resting nuclei and mitotic prophase chromosomes followed Tanaka(1977, 1971). The symbols for the description of karyotypes followed Levan *et al*.(1964).

2 Results

The two poulations studied were similar in karyomorphology of resting nuclei. In resting nuclei(Fig.2: A), numerous chromocenters were observed. The other regions were also stained well but unevenly. Thus, the resting nuclei belonged to the complex chromocenter type.

Table 1 Parameters of chromosomes in *Beesia calthifolia* from Xinning County, Hunan Province (Luo Yi-bo 945)

Chromosome No.	Diploid (2n = 2x = 16 = 8m + 4sm + 2st + 2t)			Tetraploid (2n = 4x = 32 = 16m + 8sm + 4st + 4t)		
	Relative length	Arm ratio	Type	Relative length	Arm ratio	Type
1	9.61 + 7.61 = 17.22	1.26	m	9.72 + 7.97 = 17.69	1.22	m
2	8.40 + 7.46 + 15.86	1.13	m	8.28 + 7.32 = 15.60	1.13	m
3	8.10 + 6.47 = 14.57	1.25	m	6.88 + 6.67 = 13.55	1.03	m
4	7.28 + 5.32 = 12.60	1.37	m	6.99 + 5.58 = 12.57	1.25	m
5	8.43 + 3.26 + 11.69	2.58	sm	7.28 + 3.57 = 10.85	2.04	sm
6	7.07 + 3.26 = 10.33	2.16	sm	7.15 + 3.07 = 10.22	2.33	sm
7	7.09 + 2.05 = 9.14	3.45	st	8.15 + 2.02 = 10.17	4.03	st
8	7.65 + 0.93 = 8.58	8.22	t	8.30 + 1.05 = 9.35	7.90	t

Fig. 1　Phytomicrographs of metataphase chromosomes in diploid(A, C) and tetraploid(B, D) *Beesia calthifolia* from Xinning County, Hunan Province

The two populations studied were also similar in karyomorphology of mitotic prophase chromosomes. In prophase chromosomes(Fig. 2:B), hetero- and euchromatic segments were distinguishable but their boundaries were not distinct and the heterochromatic segments were distributed in the distal and interstitial as well as proximal regions. Thus, the prophase chromosomes belonged to the interstitial type.

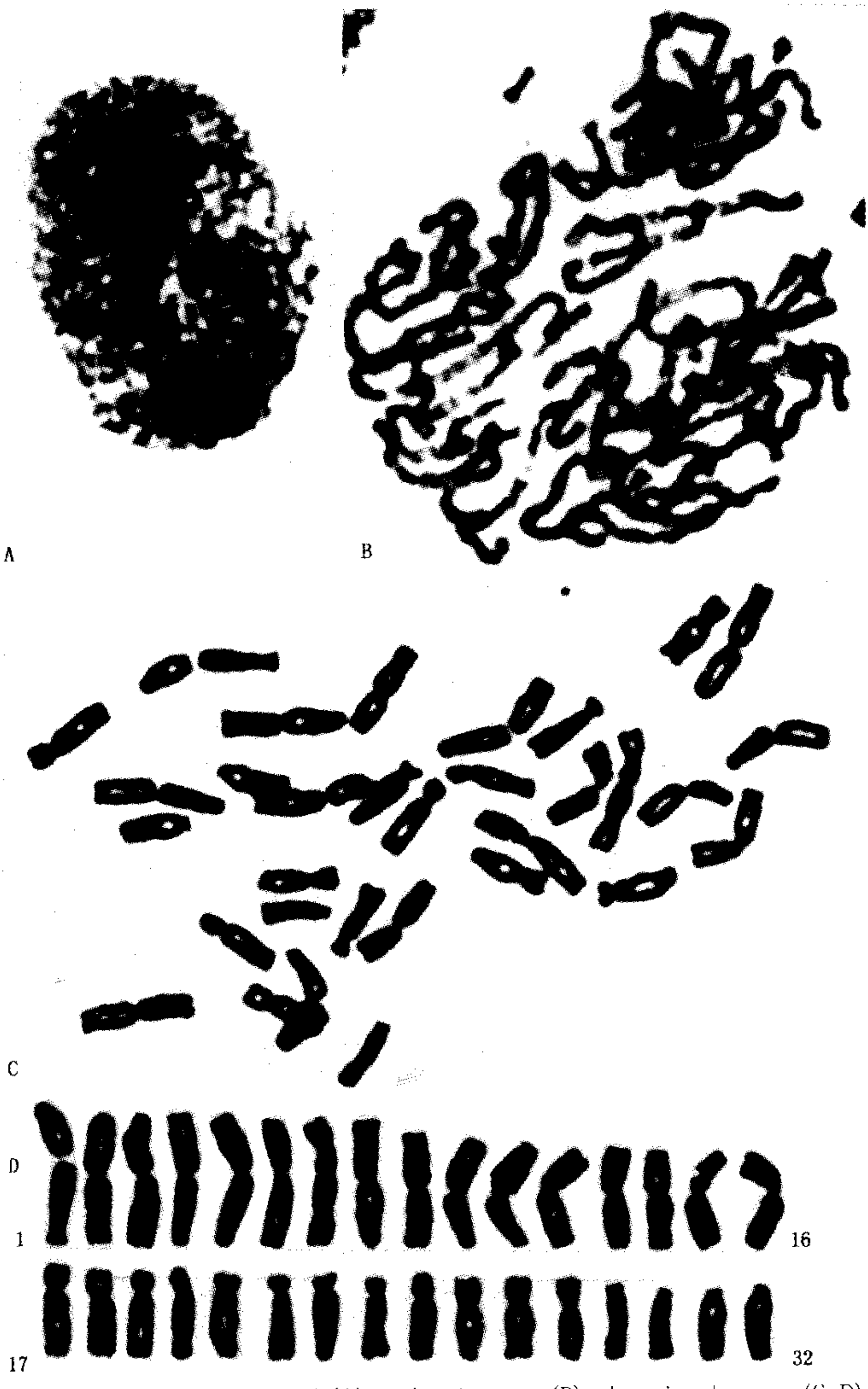

Fig. 2 Phytomicrographs of resting nuclei(A), prophase chromosomes(B) and metaphase chromosomes(C, D) in tetraploid *Beesia calthifolia* from Dali County, Yunnan Province

In the population from Xinning County, Hunan Province, 39 individuals were found to be diploids with the chromosome number of 2n = 16(Fig. 1: A, C), ranging in length from 11.89 μm to 5.93 μm, but one individual was tetraploid with the chromosome number of 2n = 32(Fig. 1 : B, D), ranging in length from 13.07 μm to 6.91 μm. The diploid plants had 8 m-, 4 sm -, 2 st- and 2 t-chromosomes(Table 1). The only tetraploid plant had 16 m-, 8 sm-, 4 st- and 4 t-chromosomes(Table 2).

All the 10 individuals of the population from Cangshan Mountain, Dali City, Yunnan Province, were found to be tetraploids with the chromosome number of 2n = 32(Fig. 2 : C, D), ranging in length from 10.34 to 5.66 μm. The karyotype was formulated as 2n = 32 = 16m + 8sm + 4st + 4t(Table 2).

Table 2　Parameters of chromosomes in *Beesia calthifolia* from Dali City, Yunnan Province (Yang Qin-er 9415)

Chromosome No.	2n = 4x = 32 = 16m + 8sm + 4st + 4t		
	Relative length	Arm ratio	Type
1	9.11 + 7.61 = 16.72	1.20	m
2	8.95 + 7.11 = 16.06	1.26	m
3	7.24 + 6.53 = 13.77	1.11	m
4	6.80 + 5.55 = 12.35	1.23	m
5	8.01 + 3.19 = 11.20	2.51	sm
6	6.92 + 3.25 = 10.17	2.13	sm
7	8.34 + 2.21 = 10.55	3.77	st
8	8.04 + 1.13 = 9.17	7.12	t

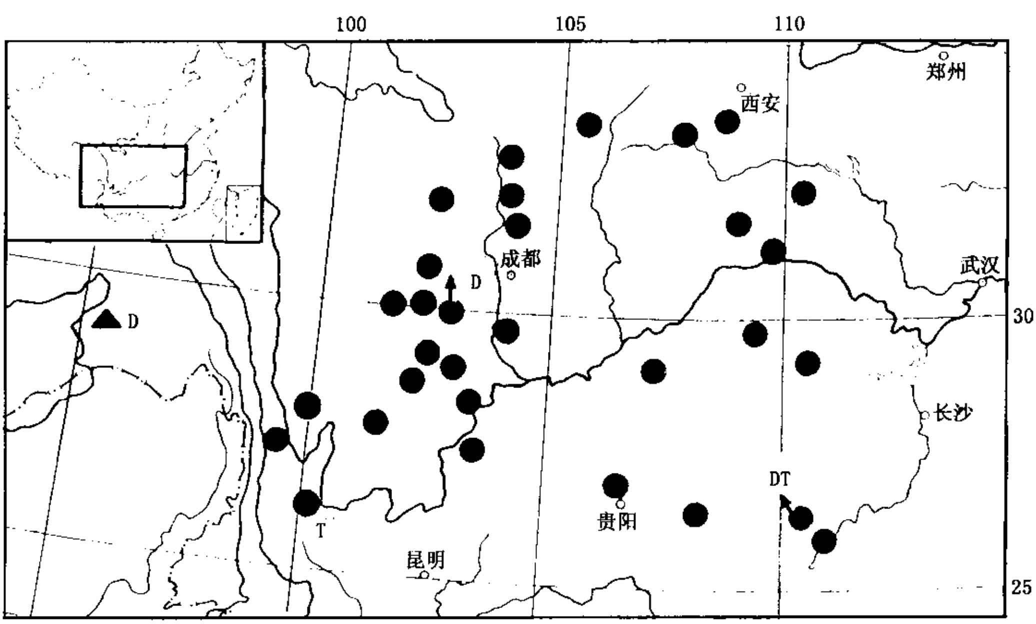

Fig. 3　Distribution of the two species in *Beesia* and their ploidy
▲ *B. deltophylla*　● *B. calthifolia*
D = diploid; T = tetraploid

3 Discussions

3.1 Karyotype of the diploid *Beesia calthifolia*

The present results of the karyotype analysis of the diploid *Beesia calthifolia* are somewhat different from those reported by Shang(1985). Shang reported the karyotype as consisting of 10 m-, 4 sm- and 2 t-chromosomes, the fifth pair having an arm ratio of 1.22. In this study, the karyotype was found to consist of 8 m-, 4 sm-, 2 st- and 2 t-chromosomes, the fifth pair of chromosomes of this species having an arm ratio of 2.58. In the karyotype of *Beesia deltophylla*, although the fifth pair of chromosomes is categorized as m-chromosomes, their arm ratio has reached 1.66. As seen from the chromosome figures in Shang's paper, the fourth pair of chromosomes should have subterminal centromeres, the satellites as recognized by Shang actually being the short arms. If so, the karyotype of the materials studied by Shang actually also consists of 8 m-, 4 sm-, 2 st- and 2 t- chromosomes, which is basically the same as that of the materials studied here. The results are further confirmed to great degree by those of the karyotype analyses of the tetraploid plants. The only tetraploid plant from the same population of Hunan as the diploid plants and the 10 tetraploid plants from the population of Yunnan all have 16 m-, 8 sm-, 4 st- and 4 t-chromosomes. More importantly, the chromosome parameters of the diploid and tetraploid plants are quite near to each other. Thus, their uniformity in karyotype constitution should not have resulted from artificial errors in the measurement of chromosomes.

3.2 Main differences of the karyotype of the genus *Beesia* and those of the other genera in the tribe Cimicifugeae

There exist some differences between the karyotypes of *B. deltophylla* and *B. calthifolia*. For example, the fifth chromosome pair in the former species belongs to m-chromosomes, even though the arm ratio has reached 1.66(Yang *et al.*, 1995), while the fifth pair in the latter belong to sm-chromosomes, with the arm ratio being over 2.00(Table 1). Furthermore, there are two very small satellites on the short arms of the eighth chromosome pair in *B. deltophylla*, while no satellites are observed on the counterparts in *B. calthifolia*. Nevertheless, basically, the karyotypes of the two species under question are very similar in comparison with the other groups in the Ranunculaceae.

Compared with the other genera in the tribe Cimicifugeae, the genus *Beesia* is karyotypically distinguished mainly in the fifth chromosome pair. This pair in *Beesia* has an arm ratio of 1.66(in *B. deltophylla*)or usually over 2.00(in *B. calthifolia*), while in the remaining genera of the tribe this pair belongs to typical m-chromosomes, with the arm ratio being around 1.20(Yang *et al.*,1993; Hasegawa, Peng, 1991; Blair, 1975; Hasegawa, 1970b; Kawano *et al.*,1966; Kurita, 1957). Thus, this pair of chromosomes may serve as the chromosomal marker of the genus *Beesia*, by which it is cytologically distinguishable from the other genera in the tribe Cimicifugeae.

3.3 Polyploidy in the genus *Beesia*

Polyploidy is very rare and might have played an unimportant role in the speciation of the tribe Cimicifugeae. Up to date, tetraploid cytotype has been reported only in *Cimicifuga foetida* from Thimphu of Bhutan(Hasegawa, 1969). So this is the second time to record a tetraploid cytotype in this tribe. Although in the 40 plant individuals from the population of Xinning County, Hunan Province, only one individual is tetraploid, all the 10 individuals from the population of Dali City, Yunnan Province, have stable chromosome number of 2n=

$4x=32$, and thus are all tetraploids. Gross-morphologically, the diploid plants show no significant differences from tetraploid ones, though there may exist some differences in their pollen grain size and stomatal size, as has been observed in *Cimicifuga foetida* by Hasegawa (1970a, 1969). *Beesia calthifolia* is widely distributed in China's northern Yunnan, Sichuan, Guizhou, northern Guangxi, western Hunan, western Hubei, southern Shanxi and southern Gansu, and northern Myanmar(Fig. 3)(Ying & Zhang, 1994; Xiao, 1979). As shown in Fig. 3, the tetraploid cytotype occurs on the southwestern margin of the distribution area of *Beesia calthifolia*, which is situated within the Hengduan Mountain region, where the seed plants in many groups show strong gross-morphological differentiation. It should be of great interest to use a cytogeographical approach to address the chromosomal differentiation of *Beesia calthifolia* in reference to its gross-morphological differentiation.

3.4 Systematic position of the genus *Beesia*

The systematic position of the genus *Beesia* has long been a controversial matter. When Balfour and W. W. Smith(1915) established this genus based on *B. cordata* Balfour and W. W. Smith, they considered that it is related to *Glaucidim* and *Hydrastis*. This viewpoint was never accepted. Hutchinson(1923) thought that *Beesia* might be actually closely allied to *Souliea* and *Cimicifuga* and established a subtribe Cimicifuginae to accommodate them, and simultaneously pointed out that *B. cordata* is a later synonym of *Cimicifuga calthaefolia* Maxim. ex Oliv.. Ulbrich(1929) agreed with Hutchinson and formally proposed that the correct scientific name should be spelled as *B. calthifolia* (Maxim. ex Oliv.) Ulbr., and pointed out that *Beesia* and *Cimicifuga* are two closely related but quite distinct genera. This opinion has been basically accepted by most of the later authors, such as Janchen(1949), Buchheim(1964) and W. T. Wang(1979). Tamura(1966), however, put *Beesia* together with *Caltha*, *Trollius*, *Megaleranthis* and *Calathodes* in the tribe Trollieae under the subfamily Helleboroideae, and regarded that *Beesia* may represent the intermediate type between the tribe Trollieae and the tribe Cimicifugeae (including *Anemonopsis*, *Souliea*, *Cimicifuga*, *Actaea* in the sense of Tamura). Very recently, Tamura (1995, 1990) made a new classification of the family Ranunculaceae. He subdivided the subfamily Helleboroideae into 4 tribes, i.e. Helleboreae(including 3 subtribes, Calthinae, Beesinae and Helleborinae), Cimicifugeae, Nigellae and Delphineae. He separated *Beesia* as an independent subtribe and put it between Calthinae(including *Caltha*, *Calathodes*, *Trollius* and *Megaleranthis*) and Helleborinae. Takhtajan(1987) had nearly the same opinion as Tamura that *Beesia* might be close to *Caltha*, *Calthodes* and *Megaleranthis*.

Karyomorphologically, *Beesia* appears to be much more closely akin to *Cimicifuga* and its allies, such as *Actaea* and *Souliea*, than to *Caltha* and its possible allies, i.e. *Calthodes*, *Trollius* and *Megaleranthis*. In chromosome size, the chromosomes of *Beesia* are very similar to those of *Cimicifuga* but significantly larger than those of *Caltha*, *Calthodes*, *Trollius* and *Megaleranthis*(Yang, 1995; Yang *et al*., 1995, 1993; Lee & Yeau, 1985; Blair, 1975; Hasegawa, 1970b; Kawano *et al*., 1966; Kurita, 1965, 1960, 1958, 1957). In chromosome morphology, although there exist some differences between *Beesia* and *Cimicifuga* and its allies as emphasized above, basically their chromosome morphology is quite similar to each other but most different from that of *Caltha*, *Calathodes*, *Trollius* and *Megaleranthis*(Yang, 1995; Yang *et al*., 1995, 1993; Lee & Yeau, 1985; Blair, 1975; Hasegawa, 1970b; Kawano *et al*., 1966; Kurita, 1965, 1960, 1958, 1957). In *Beesia* and other genera of the tribe Cimicifugeae, the four largest chromosome pairs have median cen-

tromeres, the fifth pair is also quite large and usually has median(in *Anemonopsis*, *Souliea*, *Cimicifuga*, *Actaea*) or rarely submedian centromeres(in *Beesia calthifolia*), the two moderately large pairs have submedian or subterminal centromeres depending on the species, and the smallest pair has very short but always clearly visible short arms and terminal centromeres(*Beesia*, *Anemonopsis*, *Souliea* and *Cimicifuga*), or simply has no short arms (only in *Actaea*). In the Ranunculaceae, such combinations of karyotypic characters are only found in this tribe. From karyomorphological evidence, therefore, it seems that *Beesia* might find its best systematic position in the tribe Cimicifugeae.

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摘要 本文重新检查了铁破锣的核型。来自湖南新宁的 40 个个体中，1 个个体为四倍体，其核型公式为 2n=4x=32=16 m+8sm+4st+4t；其余个体为二倍体，其核型公式为 2n=2x=16=8m+4sm+2st+2t。来自云南大理的 10 个个体全为四倍体，其核型公式为 2n=4x=32=16 m+8sm+4st+4t。据此认为商效民(1985)报道的该种的核型分析结果有误。他至少将第 4 对染色体的着丝点位置辨认错了。该对染色体不具有中部着丝点而实际上具有近端部着丝点。本文还比较了铁破锣和角叶铁破锣的核型差异，并详细讨论了铁破锣属的系统位置。

关键词 铁破锣；核型；系统位置